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Note

Allyldimethylsilyl ethers

New derivatives for the analysis of steroids by gas chromatography–mass spectrometry

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The potential of alkyldimethylsilyl groups (RMe_2Si–, R = C_2H_5, C_3H_7, or *t*-C_4H_9) to serve as important derivatives in the quantitation and identification of steroids by gas chromatography–mass spectrometry (GC–MS) has recently been demonstrated[1–4]. These applications are a direct consequence of the very intense $(M - R)^+$ ion in the electron-impact mass spectrum of this class of compounds. The relative intensity of the $(M - R)^+$ ion, for a given substance, is dependent on the nature of the alkyl (R) group and increases in the order $C_2H_5 < C_3H_7 \ll t\text{-}C_4H_9$. However, the actual rate of formation of the alkyldimethylsilyl derivatives occurs in the reverse order and although *tert.*-butyldimethylsilyl (*t*-BDMS) ethers have the best mass spectral characteristics, hindered hydroxyl groups cannot be silylated to form the respective *t*-BDMS ether[2].

This note reports an investigation on steroid allyldimethylsilyl (ADMS) ethers, which are found to have mass spectral properties comparable to those of *t*-BDMS ethers, but are also readily formed from hindered hydroxyl groups.

EXPERIMENTAL

Steroids were obtained from Steraloids (Pawling, N.J., U.S.A.). Allyldimethylsilyl chloride was purchased from P.C.R. (Gainesville, Fla., U.S.A.).

Allyldimethylsilyl ethers

ADMS ethers of reference steroids were prepared by allowing the steroid (<0.5 mg) to react with 100 μl of allyldimethylsilyl chloride [1 *M* in N,N-dimethylformamide (DMF)] and 100 μl of imidazole (2 *M* in DMF) for more detail, see Table I. The reaction products were isolated by filtering through Sephadex LH-20, as recently described by Kelly and Taylor for the corresponding *t*-BDMS ethers[4].

Gas chromatography–mass spectrometry

GC–MS was carried out using an AEI MS-30 mass spectrometer (22.5 eV),

TABLE I

METHYLENE UNIT VALUES AND MAJOR MASS SPECTRAL FRAGMENTS OF STEROID ADMS ETHERS

ADMS[a]	*MU*[d]	*Mass fragments*[e]
3α-Hydroxy-5α-androstan-17-one	27.00	347[f] (35), 271[g] (100)
3β-Hydroxy-5-androsten-17-one	28.00	345[f] (100), 155[h] (8)
17β-Hydroxy-4-androsten-3-one	28.92	345[f] (100), 269[g] (9)
3β-Hydroxy-5-pregnen-20-one	29.54	373[f] (100), 155[h] (17)
3α,11β-Dihydroxy-5β-androstan-17-one[b]	30.92	461[f] (4), 385[g] (22), 175 (100)
20α-Hydroxy-4-pregnen-3-one	31.06	373[f] (92), 75 (100)
5β-Pregnane-3β,20β-diol	31.54	475[f] (14), 143[i] (100), 103[j] (92)
5-Pregnene-3β,17α,20α-triol[c]	34.94	485[k] (39), 173[l] (100), 143[i] (16)

[a] Formed by reaction at room temperature for 1 h, unless otherwise stated.
[b] 1 h at 75°, partial reaction after 5 h at room temperature.
[c] 4 h at room temperature or 1 h at 75°.
[d] Column, 1.5 m × 2 mm I.D., packed with 1% OV-101 on Gas-Chrom Q; $T = 250°$.
[e] Expressed as M/z value with relative intensity in parentheses.
[f] $(M - 41)^+$.
[g] $(M - 41 - 76)^+$.
[h] $(C_3H_5)(CH_3)_2Si\text{-}O^+{=}CH\text{–}CH{=}CH_2$.
[i] $CH_3CH{=}O^+\text{–}Si(CH_3)_2(C_3H_5)$.
[j] $C_2H_5\text{–}O^+{=}Si(CH_3)_2$.
[k] $(M - 143)^+$.
[l] $(CH_3)_2Si{=}O^+\text{–}Si(CH_3)_2(C_3H_5)$.

equipped with a DS-50 data system, and interfaced to a Pye 104 gas chromatograph (column, 1.5 m × 2 mm I.D., 1% OV-101, 250°) via a single-stage glass jet separator (S.G.E.).

RESULTS AND DISCUSSION

From the data in Table I it can be seen that the reagent combination employed is a powerful silylating mixture which will silylate unhindered hydroxyl groups in approximately 1 h at room temperature. The more hindered tertiary 17- and secondary 11-hydroxyl groups require longer reaction periods or higher temperatures. At these higher reaction temperatures, however, silyl-enol formation of α,β-unsaturated ketones becomes a competitive side-reaction, which unfortunately cannot be forced to completion[5]. Extended reaction times at high temperature also cause the gradual formation of other unidentified products.

The ADMS ethers have excellent GC properties, and for mono-ADMS steroids show an average methylene unit (*MU*) increment of 1.95, in comparison to their respective trimethylsilyl analogues.

The mass spectra of these derivatives, except the steroid tri-ADMS ethers, all show a very intense $(M - 41)^+$ ion (Table I). Some of the ADMS ethers also display mass fragment ions which are diagnostically characteristic of the position and or proximity of the silyl groups, *e.g.*, M/z 173 (1,2-di-ADMS), 143 (20-ADMS), 155 (5-ene-3β-ADMS).

The percentage of the total ion current carried by the $(M - 41)^+$ ions of steroid mono-ADMS ethers is usually >20% (testosterone-ADMS = 33%), which

implies these derivatives will be particularly suited to the quantitation of trace amounts of steroids by mass fragmentography.

Work is now in progress to extend the use of these derivatives to those steroids which contain both hindered hydroxyl and readily enolizable ketonic groups.

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